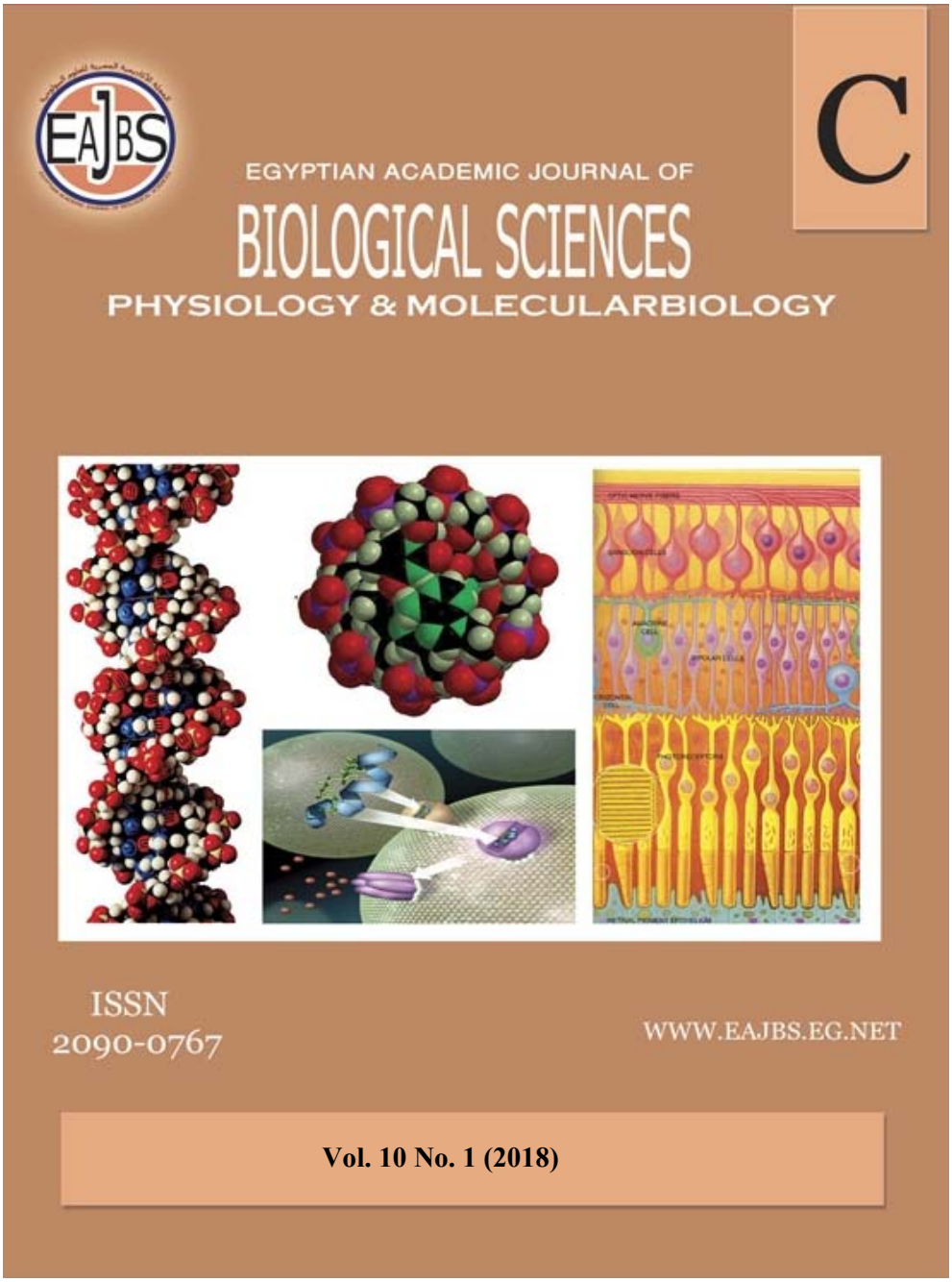

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Genetic Variation and Phylogenetic Relationship among four Parrotfishes (genus Scarus) in Hurghada, Red Sea Coast, Egypt Based on RAPD Markers

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ABSTRACT

Random amplified polymorphic DNA (RAPD) fingerprints were obtained for 4 Parrotfishes (genus Scarus) namely, *Scarus collana* Rüppell 1835; *Scarus frenatus* Lacepède 1802; *Scarus (Chlorurus) sordidus* Forsskål 1775 and *Scarus niger* Forsskål 1775 using RAPD-PCR typing technique. A total of 111 bands (ranging from 200 to 2000 base pair) were produced, 13 monomorphic (common) and 98 polymorphic (88.29% level of polymorphism). The *Scarus frenatus* recorded highest band frequencies among studied species. Under phenetic approaches "clustering using unweighted pair group method average (UPGMA) based on Nei-72 genetic distance, principal coordinate analysis (PCOA), and neighbour joining (NJ) based on pairwise mean character differences", the studied scarids formed two groups as sister taxa and most likely to have the most common ancestor. There is a close genetic relatedness among group members [*Scarus collana*/*Scarus niger* and *Scarus frenatus*/*Scarus sordidus*]. According to parsimony (Cladistic) analysis, RAPD markers obtained are reliable and phylogenetically instructive. The four parrotfishes are related, but *S. frenatus*, *S. sordidus*, and *S. niger* have a close evolutionary relationships. Splitting of *Scarus collana* as basal group suggested a common ancestor having unique features (synapomorphies) be inherited in the studied parrotfishes. This study's results possibly provide useful information about the genetic variation and phylogenetic relationships among parrotfishes.

INTRODUCTION

Fishes are diverse species within the animal kingdom including about 50% of recognized vertebrates. Fishes are a major protein dietary sources for world's population (FAO 1997 and FAO 2000)**.**

Fishes include more than 32,000 species placed in about 482 families, from them ~ 13,000 marine fish species exist (Nelson, 1994**).** Red Sea harbors over 1,000 fish species, mostly associated with coral reefs and display diverse colour patterns

(juveniles), or noticeably colour differences during sexual development (wrasses, Labridae) (Randall, 1982**).**

Parrotfishes (with beak-like mouths) are a distinctive group of coral reef herbivores exhibiting multiplicity in cranial morphology and function (Wainwright *et al.,* 2004; Westneat, 1995; Westneat and Alfaro, 2005) and play important role in enhancing reef resilience (Bellwood *et al.,* 2003; Burkepile and Hay, 2008; Cheal *et al.,* 2010). Taxonomically, parrotfishes include about 95 species, formerly assigned to a family-level taxon (Scaridae) but recently reclassified as scarine labrids (subfamily Scarinae, family Labridae). Nevertheless, others still prefer to maintain them as a family-level taxon (Bellwood, 1994; Choat and Bellwood, 1998; Westneat and Alfaro, 2005; Randall, 2007**).**

Random amplified polymorphic DNA (RAPD) involves the use of a solitary short (arbitrary) primer in a PCR reaction, resulting in the amplification of many discrete DNA amplicons (Williams *et al.,* 1990; Welsh and McClelland, 1990).

Randomly distributed loci inside a genome can be detected by RAPD which facilitated the development of a variety of genetic markers. These RAPD markers/loci are considered as dominant genetic markers inherited in a Mendelian fashion (Rothuizen and Van Wolferen, 1994). RAPD is most used fingerprint DNA-based method for species identification and taxonomy among organisms. It has been widely applied in several studies including animals, fishes and plants (Dinesh *et al.,* 1993; Liu *et al.,* 1999; Ali *et al.,* 2004; Callejas and Ochando, 1998; Geertjes *et al.,* 2004; Wang *et al.,* 2005; Singh *et al.,* 2009; Sayed, 2012).

The classification, genetic diversity and the evolutionary history of parrotfishes remain debatable. There is a need for more molecular phylogenetic studies to resolve the taxonomic related problems of parrotfishes. Therefore, using molecular tools based-DNA fingerprints may provide insights into their evolutionary relationships, genetic diversity and species richness. The present work aims to use RAPD-PCR assay to investigate the genetic variation, and phylogenetic relationship among four parrotfishes species (genus scarus): *Scarus collana* Rüppell 1835; *Scarus frenatus* Lacepède 1802; *Scarus (Chlorurus) sordidus* Forsskål 1775 and *Scarus niger* Forsskål 1775.

MATERIALS AND METHODS

Taxon Sampling :

Parrotfish species (Fig. 1) were obtained from Hurghada fish market (Halaka), Red Sea Coast, Egypt in May 2016 (marine water captured economic fishes). After collection by the author, the fishes were kept on ice, then brought to the laboratory. Fish were sorted down to species level (Bellwood 2001; FishBase 2016). Tissue samples (muscle, fins and scales) were removed and individually ethanol preserved at -20°C for genomic DNA extraction and future analyses.

Fig. 1: Photographs of the four Parrotfishes (genus scarus) species under study obtained from Hurghada fish market (Halaka), Red Sea Coast, Egypt in May, 2016. These are *Scarus collana* Rüppell 1835; *Scarus frenatus* Lacepède 1802; *Scarus (Chlorurus) sordidus* Forsskål 1775 and *Scarus niger* Forsskål 1775.

Fish Genomic DNA Extraction:

Genomic DNA from fish samples was extracted using approximately 30 mg of muscle tissue specimens by the EZ-10 spin column genomic DNA extraction kit for animal tissue (Bio Basic Inc., Canada) according to the manufacturer's instructions. DNA samples were stored at −20 °C until used. DNA concentration and purity were estimated under spectrophotometric UV absorption at A260 and A280 and 1% agarose gel.

RAPD PCR Analysis and DNA Amplification:

RAPD-PCR was performed as described by Williams *et al.,* (1990) in reaction volume of 25 µl with final concentration containing approximately 50 ng genomic DNA of each sample, 1.0× pre-mixed OnePCRTM 2X (GeneDireX Inc, USA), and 10 pM each primer [A-01, A-02, A-03, A-04, A-05, A-06, A-07, A-08, A-09, A-10, A-11, and A-12] (Bio Basic Inc, Canda). The reaction was performed in thermocycler (PEQLAB Biotechnologie GmbH) under the following cycling settings consisting of initial denaturation at 95°C for 2 min, followed by 45 cycles (94°C for 1 min, 36°C for 1 min and 72°C for 2 min), then one cycle of final extension at 72°C for 10 min. Amplified products were separated in 1.5% (w/v) agarose in 1× TAE buffer (0.40 mM Tris, 0.20 mM acetate, 2 mM EDTA pH 8). Gels were photographed with Elttrofor M20 SaS Photo-Gel System (Italy) with Nikon Coolpix LB40 digital camera. Size of DNA bands were determined with 100 bp DNA ladder (0.1 µg/µl, Solis BioDyne, Estonia).

Data Analysis:

For RAPD polymorphism analysis, amplified DNA bands were monitored and identified from RAPD images by the Totallab 1D v12.2 software (TotalLab Ltd., Newcastle-upon-Tyne, UK). DNA fingerprints were scored for the presence (1) or absence (0) of similar-sized DNA bands in order to generate a binary data matrix. For each primer used in the RAPD assay, the total number of scored bands, number of

polymorphic/monomorphic bands were recorded.

Phenetic Analysis of RAPD data:

For phenetic analysis of RAPD data, three approaches were used. **1]** The POPGENE program version 1.32 (Yeh *et al.,* 1999) was used to calculate Nei's original measures of genetic identity and genetic distance (Nei, 1972)**.** RAPD data clustered using the unweighted pair group method average "UPGMA" (Sneath and Sokal 1973) based on the Nei-1972 genetic distance and displayed with the Molecular Evolutionary Genetics Analysis (MEGA v6) software (Tamura *et al.,* 2013**). 2]** To provide a dimensional graphical representation for fish samples under study, the RAPD data was analysed by the principal coordinate analysis (Gower, 1966**)** using the PAST program (Hammer *et al.,* 2001) based on the computed Jacquard's coefficient (Jaccard 1908**). 3]** RAPD data binary matrix of four taxa and 111 characters/bands (absence/presence 0/1) was subjected to the neighbour joining clustering (Saitou and Nei 1987) under distance criterion using PUAP version 4.0a150 (Swofford, 2001) with a distance measure based on the mean character differences. The reliability of NJ tree was validated by bootstrap analysis with 500 replicates as all 111 characters were included.

Cladistic Analysis of RAPD Data :

The RAPD data in a binary matrix of four taxa and 111 characters/bands (absence/presence 0/1) was analysed under the maximum parsimony criterion using the *PAUP* version 4.0a150 software (Swofford, 2001**)** with heuristic search options for unordered and equally weighted characters. Heuristic searches were performed using random taxon addition, tree bisection-reconnection branch swapping (TBR) for most-parsimonious trees, accelerated transformation (ACCTRAN) character optimization, and treated missing gaps. To measure the degree of support for each individual branch, bootstrap analyses (Felsenstein 1985) of 500 replications were performed. Tree statistics including consistency index (CI) (Kluge and Farris 1969)**,** homoplasy index (HI) and retention index (RI) (Farris 1989) were computed.

RESULTS

Assessing the Genetic Polymorphism:

Twelve 10-mer primers were screened for generating reliable fingerprints patterns and assessing polymorphism of the parrotfishes under study (Table 1). A hundred and eleven bands were produced with an average of 9.25 bands per primer, from them 13 bands with 11.71% level of monomorphism were common among the studied species. Among the amplicons 98 were polymorphic exhibits level of polymorphism of 88.29%. The average number of polymorphic fragments/primer of 8.17 was calculated based on NPBands/12 primers.

All primers generated multiple banding patterns of 6 to 11 polymorphic amplified DNA bands ranging in size from 200 to 2000 bp as compared to a 100 bp DNA Ladder (Solis Bio Dyne). Primers A-04, A-06 and A-11 detected a maximum number of 11 amplicons each, while the minimum number of 6 fragments was amplified with primer A-12. The highest number of polymorphic bands (11) was obtained with primers A-04 and A-11, while the lowest ones (4) was obtained with primer A-12.

The band frequency per species were 0.4865, 0.5586, 0.5045 and 0.5315 for *S.collana*, *S. frenatus*, *S. sordidus* and *S. niger* respectively while band frequency per primer ranged from 0.0541 to 0.0991 as shown Fig. 2. The RAPD genotyping banding profile generated by all primers are presented in Fig. 3

Table 1: Characteristic of RAPD primers used. Number of Amplified Bands per Species (NABands/Species), Band Frequency per primer (Band Freq/Primer), Band Frequency per Species (Band Freq/ Species), Total number of Amplified Bands (TNABands), Number of polymorphic bands (NPBands), Number of monomorphic bands (NMBands) Polymorphism Percentage (POL%), and Range of amplified fragment in base pair (RAF [bp]).

Primer Code	Sequence	NABands/Species				TNA Bands	Band Freq/ Primer	NP Bands	NM Bands	% POL	RAF [bp]
	5'---------3'	*Scarus collana*	*Scarus fernatus*	*Scarus sordidus*	*Scarus niger*						
A-01	CAGGCCCTTC	4	7	5	5	9	0.0811	7	2	77.78	400-2000
A-02	TGCCGAGCTG	5	4	4	6	8	0.0721	6	2	75.00	300-1500
A-03	AGTCAGCCAC	4	8	6	5	10	0.0901	9	1	90.00	400-2000
A-04	AATCGGGCTG	5	6	4	4	11	0.0991	11	0	100.00	200-1200
A-05	AGGGGTCTTG	3	5	3	4	7	0.0631	6	1	85.71	500-1200
A-06	GGTCCCTGAC	7	6	5	7	11	0.0991	8	3	72.73	200-1900
A-07	GAAACGGGTG	4	5	6	5	10	0.0901	9	1	90.00	200-1600
A-08	GTGACGTAGG	5	3	5	6	10	0.0901	9	1	90.00	200-1500
A-09	GGGTAACGCC	4	4	4	4	8	0.0721	8	0	100.00	400-1500
A-10	GTGATCGCAG	4	5	4	4	10	0.0901	10	0	100.00	200-1200
A-11	CAATCGCCGT	5	4	7	5	11	0.0991	11	0	100.00	400-2000
A-12	TCGGCGATAG	4	5	3	4	6	0.0541	4	2	66.67	400-1000
Total		54	62	56	59	111		98	13	88.29	
Band Freq/ Species		0.4865	0.5586	0.5045	0.5315						

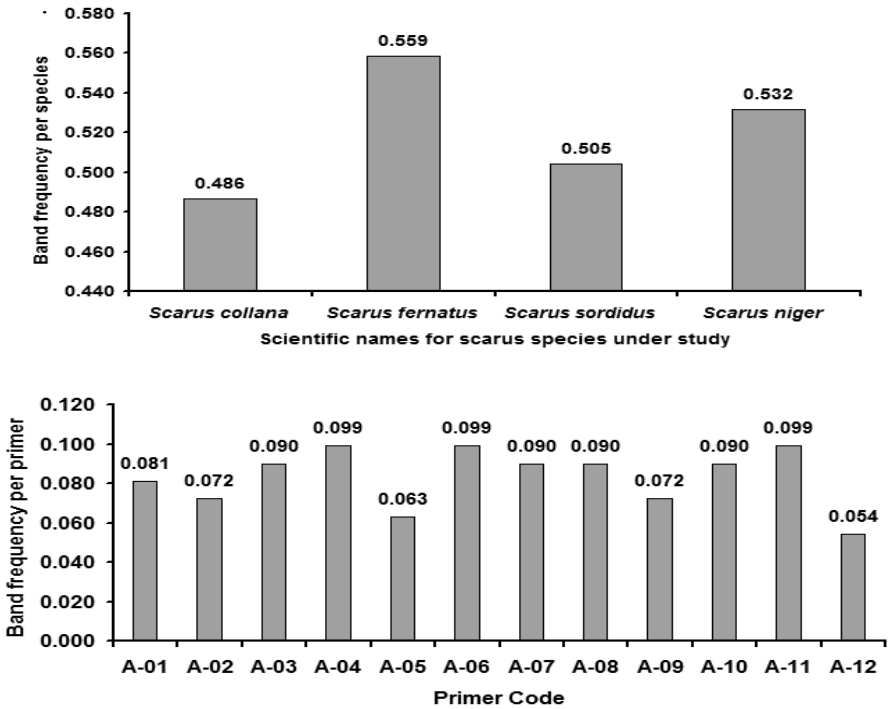

Fig. 2: Band frequencies recorded for the Parrotfishes, *Scarus collana*, *Scarus frenatus*, *Scarus.*(*Chlorurus*) *sordidus*, and *Scarus niger* (Above) and per the 12 deca-nucleotide primers (Below)**.**

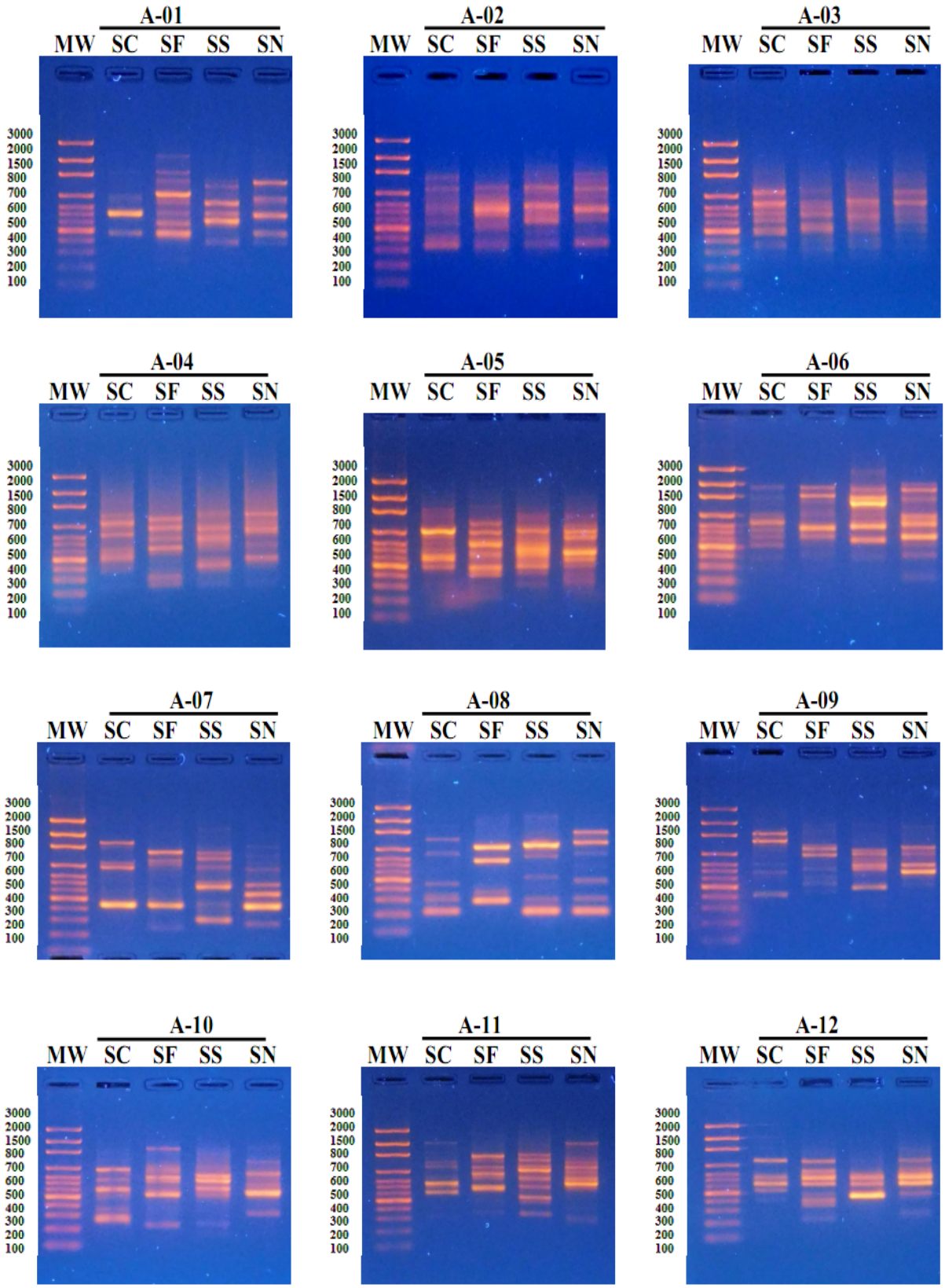

Fig. 3: RAPD-PCR fingerprints obtained from four Parrotfishes species generated by the 12 10-mer primers. **MW**: Molecular weight (100-3000 base pair), **SC**: *Scarus collana*, **SF**: *Scarus frenatus*, **SS**: *Scarus sordidus* and **SN**: *Scarus niger*

Phylogeny Analyses and Genetic Relationships Phenetic Analysis of RAPD Data :

According to the Nei-72 genetic distance matrix (Table 2), the genetic distance values ranged from 0.5506 to 0.7777. These results suggest that genetically closest scarid species are *S. collana and S. niger* (value of 0.5506); *S.sordidus* and *S.niger* (value of 0.5824) followed by *Scarus frenatus* and *Scarus sordidus* (value of 0.6665). While the most genetically distant scarid species are *Scarus collana* and *Scarus sordidus* (value of 0.7777) followed by *Scarus collana* and *Scarus frenatus* (value of 0.7392

Table 2: The Nei's original measures of genetic identity and genetic distance (Nei, 1972) computed for RAPD data binary matrix. Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

	Scarus collana	***Scarus frenatus***	***Scarus sordidus***	***Scarus niger***
Scarus collana	-	0.4775	0.4595	0.5766
Scarus frenatus	0.7392	-	0.5135	0.4144
Scarus sordidus	0.7777	0.6665	-	0.5586
Scarus niger	0.5506	0.8809	0.5824	-

The UPGMA clustering based on the corresponding Nei-72 distance matrix of genetic produced a tree (Fig. 4) which clearly distinguished two groups: *Scarus frenatus* and *Scarus sordidus* grouped into a single cluster as sister group joined at node **N1**, while *Scarus collana* and *Scarus niger* grouped into another cluster joining at node **N2** and both groups are most likely to have a common ancestor (see **N3**).

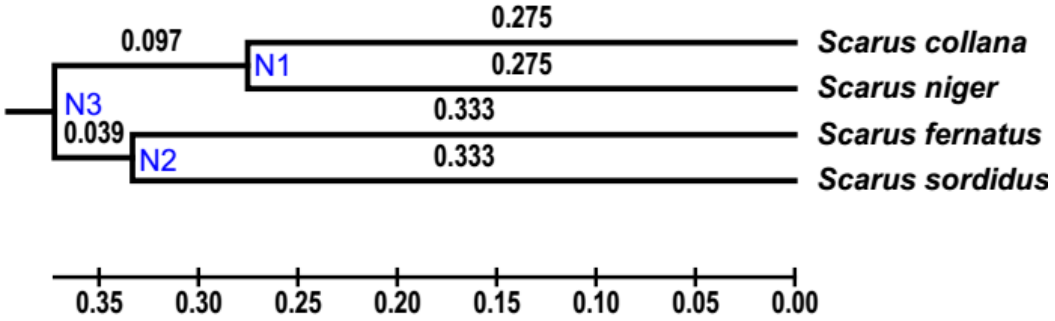

Fig. 4: Dendrogram illustrating the genetic relationships among the four studied Parrotfishes species based on estimates of Nei's (1972) genetic distances for RAPD data (Table 2) and constructed using the UPGMA method of clustering**.** Branch lengths (1:100) and tree nodes (N1, N2 and N3) are shown.

To assess the result obtained by the UPMGA, the PCoA analysis graphically demonstrated the relationships between the scarid species under study (Fig. 5) where the first two principal coordinate axes accounted for 42.088% (Eigenvalue 0.26596) and 36.834% (Eigenvalue 0.2362) from the total variation respectively. The more similar species are *Scarus collana* and *Scarus niger* (cluster 1) followed by *Scarus frenatus* and *Scarus sordidus* (cluster 2), while those less similar species are *Scarus frenatus* and *Scarus niger*, then *Scarus collana* and *Scarus sordidus* as shown by the minimum spanning tree line.

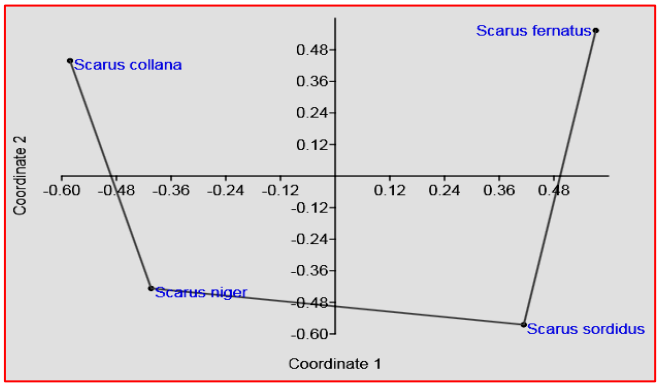

Fig. 5: The principal coordinate analysis as a dimensional graphical view illustrating the relationships among the four Parrotfishes species under study**.**

The NJ clustering of RAPD data based on pairwise distances between studied species (Table 3) produced a tree (Fig. 6) displaying the degree of relatedness among the scarid species under study. As shown in Table 3, the mean values ranged from 0.42342 to 0.58559 reflecting such relationships. The mid point rooting of such tree for most branch lengths resulted in a concurrent tree similar to the UPMGA tree (Fig. 4) except for branch lengths which clearly distinguished the two clusters (sisters clade) of the studied scarid species. Additionally, bootstrap analysis with 500 replications produced a 50% majority rule consensus tree with branches reaching a bootstrap support of 72% between *Scarus frenatus* and *Scarus sordidus* (Fig. 7).

Table 3: Pairwise distances among the studied scarid species. Below diagonal; the total character differences. Above diagonal; the mean character differences

	Scarus collana	***Scarus frenatus***	***Scarus sordidus***	***Scarus niger***
Scarus collana	-	0.52252	0.54054	0.42342
Scarus frenatus	58	-	0.48649	0.58559
Scarus sordidus	60	54	-	0.44144
Scarus niger	47	65	49	-

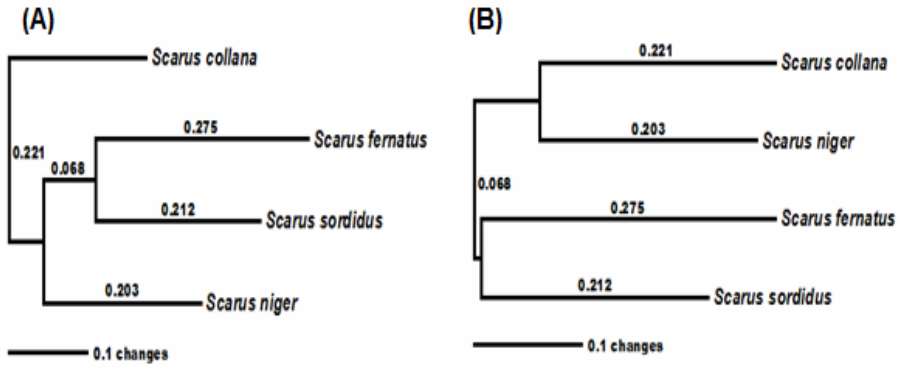

Fig. 6: The neighbour joining tree generated from distance measures based on the mean character differences computed by PAUP for RAPD markers as all 111 characters are included (A). The mid point rooting for the same tree (B).

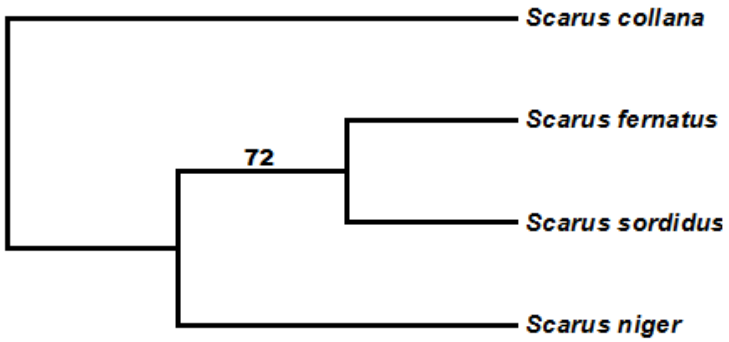

Fig. 7: The 50% majority rule consensus tree of fish samples used in the neighbour joining analysis of RAPD markers under a bootstrap analysis with 500 replicates. The bootstrap value is shown as percentage and branch lengths have no significance.

Cladistic Analyses of RAPD Data:

Parsimony analysis using heuristic searches revealed 13 constant characters out of 111 characters, 59 variable parsimony-uninformative characters and 39 variable parsimony-informative characters. Upon the completion of the heuristic search, a parsimonious tree with 119 steps was constructed (Fig. 8) showing the cladistic relation among the studied parrotfishes, where *Scarus frenatus* and *Scarus sordidus* grouped in single clade and *Scarus niger* is related to them, while *Scarus collana* was split up as out-group. When such parsimonious tree rooted at the midpoint (for most branch lengths), a concurrent tree similar to the UPMGA tree (Fig. 4) was produced except for branch lengths which clearly showed the two groups (sisters clade) (also see Fig. 6).

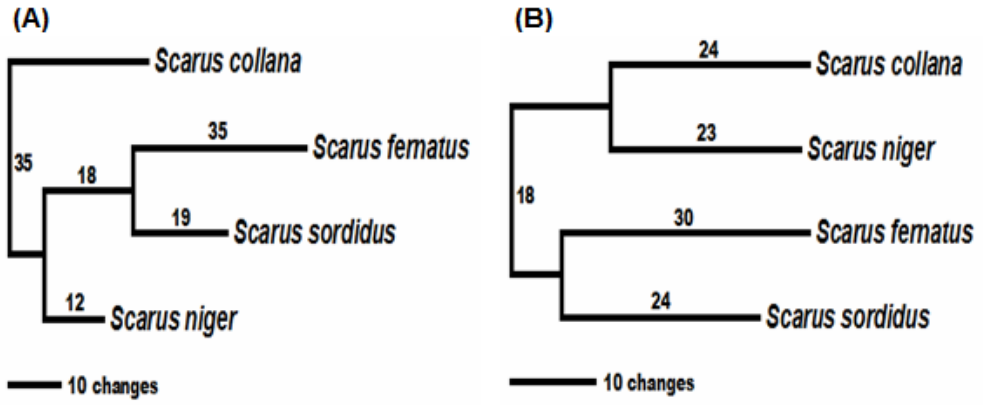

Fig. 8: The most parsimonious tree with 119 steps produced using heuristic search with statistic support values of CI=0.8235, RI=0.4615 and HI=0.1765 (A), while the mid point rooting for the maximum branch length for the same tree is shown in (B).

The bootstrap analysis with 500 replications produced a 50% majority-rule consensus unrooted tree that shows a branch of 18 steps appearing in 72% of the bootstrap replicates (>50% of replicates) which separates both the *Scarus frenatus* and *Scarus sordidus* genotypes from other genotypes presented (Fig. 9

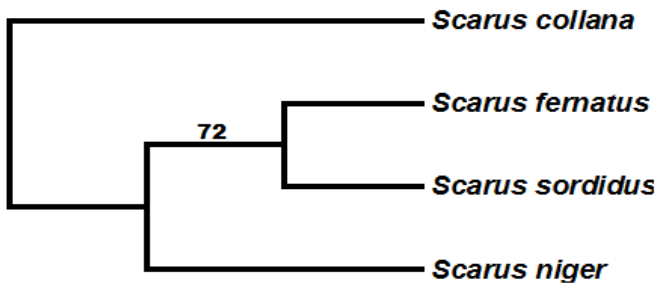

Fig. 9: The 50% majority rule consensus tree after bootstrap analysis with 500 replicates of the full heuristic search. Bootstrap values are indicated as percentages and branch lengths are unweighted

The tree consistency index, homoplasy index and retention index were 0.824, 0.176, and 0.462 respectively (Table 4). As shown in the table, a higher consistency index (CI) value of 0.8235 indicates that the configuration of the characters is supportive of the tree under maximum parsimony while the retention index (RI) value of 0.4615 is closer to 0 thus reflecting that the tree presents the best amount of synapomorphic characters. Furthermore, the homoplasy index (HI) value of 0.1765 mirrors the homoplasy level.

Table 4: Summary of statistics of the parsimonious tree with 119 steps

Tree length	119.00
Consistency index (CI)	0.8235
Homoplasy index (HI)	0.1765
CI excluding uninformative characters	0.6500
HI excluding uninformative characters	0.3500
Retention index (RI)	0.4615
Rescaled consistency index (RC)	0.3801

DISCUSSION

Herein, the RAPD markers were used to detect the genetic discrepancy among four parrotfishes (genus Scarus) species. The molecular DNA-based techniques including RAPD showed to be valuable for studying the taxonomic relationships, genetic diversity down to species levels (Demeke *et al.,* 1992) and for species identification (Callejas and Ochando, 1998; Lakra *et al.,* 2007).

The RAPD-based genetic variation among genotypes of four scarid species showed the presence/absence of RAPD band (locus/marker) of matching molecular weight suggesting their interspecies genetic alterations. Results obtained here showed a high level of polymorphism of 88.29 % (98 bands) and a low level of monomorphism of 11.71 % (13 bands) among the scarid species under study. All primers produced discrete visible number of DNA bands since the use of short arbitrary primers in a PCR reaction would be very useful in deciphering polymorphic genetic markers. These markers are in the genome of the different taxa within the coding or non-coding regions and would be selectively amplified depending on the

presence of complementary primer binding sites (Williams *et. al.,* 1990; Welsh and McClelland, 1990; Hunt and Page, 1992**).**

The band/marker frequency per species were 0.4865, 0.5586, 0.5045 and 0.5315 for *Scarus collana*, *Scarus frenatus*, *Scarus sordidus* and *Scarus niger* respectively, among them the highest band frequency of 0.5586 was recorded for *Scarus frenatus* which suggested its higher rate of heterozygosity that could be a reason for the species successful adaptations to wide range of habitats.

Nei's assumption express the influence of genetic drift and mutation on genetic disparity. A Nei's closely related distances between *Scarus frenatus* and *Scarus sordidus* as well as between *Scarus collana* and *Scarus niger* obtained from RAPD data using UPMGA positioned the four genotypes in two sister taxa groups as sister clade with a common node **(N3)** in the phylogenetic tree (Fig. 4) that indicated more genetic makeup resemblance and close relation between individuals of each cluster as well as the 4 genotypes shown to have common ancestor. The PCoA analysis (Fig. 5) resolved the same two major groups of taxa demonstrating species level similarities [*Scarus collana*/*Scarus niger* and *Scarus frenatus*/*Scarus sordidus*] which are placed close together while less similar species [*Scarus frenatus*/*Scarus niger* and *Scarus collana*/*Scarus sordidus*] are further apart, such species grouping could effected by their patterns of genetic variations take place. Similar results observed for the tree generated by the neighbouring joining method suggested that *Scarus collana* shared a common ancestor with *Scarus niger* and with both *Scarus sordidus*, and *Scarus frenatus*, since those located in a single clade are supported with 72% bootstrapping (Figs. 6 and 7).

The parsimonious tree of 119 steps of the heuristic searches (CI=0.8235, RI=0.4615 and HI=0.1765) could reveal the evolutionary relationships within the species studied, where *Scarus frenatus* and *Scarus sordidus* split from the same node as sister group (monophyletic group) indicating that they are the closest relatives as evidenced in the parsimony analyses (72% bootstrap support) (Fig. 9). In the meanwhile, *Scarus niger* was positioned as a sister clade to them, while *Scarus collana* split as an outgroup. The splitting of *scarus collana* could clarify that all other species are more closely related to each other than they are to the *Scarus collana.* Therefore, the position of *Scarus collana* as a basal group suggested an evolutionary linage that possess unique features (synapomorphies) deposited within the genetic complement of fish species under study. As reported by Choat *et al.,* (2012), *Scarus collana* is one of two basal lineages common in the shorelines of northern Indian Ocean and Red Sea. Even so, the rooting of the parsimonious tree of 119 steps at the midpoint of the maximum branch length resulted in a concurrent tree similar to the UPMGA tree (Fig. 4). Despite the branch lengths, the tree clearly showed the two groups (sisters clade) of the scarid species studied here. The evolution of RAPD bands/markers used here in the NJ and MP analyses appears to be reliable and may provide insights into the evolutionary linages for the parrotfishes species under study.

Results obtained showed similar clade placements of studied parrotfishes to those previously reported using different approaches. Westneat and Alfaro, (2005) reported that *Scarus* (Chlorous) *sordidus* and *Scarus frenatus* are closely related positioned in a clade with another species *scarus dimidiatus* (not included in this study) sharing a common ancestor giving a sub clade to the scarines. Saad *et al.,*

(2013**)** utilized the ISSR technique to reconstruct phylogenetic trees for some parrotfishes suggesting the placement of *Scarus frenatus and scarus (chlorous) sordidus* in a sub clade, while *Scarus niger* was placed as sister clade to them. The results of this study and those of Saad *et al.,* (2013) reflected the close relatedness of the species, but the current study suggested that *Scarus frenatus* could possess a high level of genetic polymorphism which results in its successful adaptation. Smith *et al*., (2008) in their constructed tree pointed to that the *Scarus* clade 4 is composed of eight Indo-West Pacific taxa which formed due to the *Scarus* adaptive radiation process, among them *Scarus frenatus and Scarus niger* which showed a close clade position to other divergent scarus species.

The work carried out by Streelman *et al.,* (2002) indicated the break between two clades of parrotfishes of genera of seagrass and those of coral reef based on the DNA sequence of four loci [the nuclear Tmo-4C4 gene, the mitochondrial cytochrome b, and ribosomal 12S and 16S genes]. This was in agreement with Schultz (1958) dividing them to Scariane (reef) and Sparisomatine (grass), and contradicted with Bellwood (1994**).** Beside the break process that occurred about 42 million years ago, the accumulation process for several kinds of mutations would be occurred. Such mutations may later and at certain specific time become part of the individual's genome that result in accumulation of several genetic variations leading to more genetic biodiversity and adaptation. This may explain the opinion of Bellwood (1994**)** to maintain the parrotfishes as a family-level taxon.

In conclusion, the results of the current study showed the sensitivity and effectiveness of RAPD assay in fish phylogenetic studies to the species level and generated distinctive DNA fingerprints for the four parrotfish species under study. The phenetic and parsimony measures applied here suggested similar clade placements of the four scarus species studied. Based on the phenetic analysis, the 4 parrotfishes (genus Scarus) formed two groups as sister taxa sharing a common ancestor. There are genetic relatedness among the group members (*Scarus collana*/*Scarus niger* and *Scarus frenatus*/*Scarus sordidus)*. According to parsimony (Cladistic) analysis, the RAPD markers obtained here are reliable and phylogenetically instructive. The four species are related, however *Scarus frenatus*, *Scarus sordidus*, and *Scarus niger* have a close evolutionary relationship. The splitting of *Scarus collana* as a basal group suggested a common ancestor that possessed unique features (synapomorphies) existing in the studied parrotfishes. The results of this study possibly will support understanding the genetic diversity and taxonomic structures of that controversial taxon of parrotfishes.

The future direction of this work would be focused on further phylogenetic analyses including the application of additional polymorphic markers such as specific genes (e.g cytochrome b and 16s rDNA).

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ARABIC SUMMARY

التباين الوراثي والعلاقة التطورية بين أربعة انواع من أسماك الببغاء (جنس Scarus) ، البحر الآحمر،الغردقة، مصر استنادا إلى علامات التضاعف العشوائي متعدد الأشكال (RAPD)

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تم دراسة التباينات الوراثية لأربعة انواع من أسماك الببغاء (جنس Scarus) هى *Scarus frenatus* Lacepède 1802، *Scarus* (Chlorurus) *sordidus* Forsskål 1775، *Scarus collana* Rüppell 1835، و كذلك *Scarus niger* Forsskål 1775 بواسطة التضاعف العشوائي (RAPD) و ذلك باستخدام البادئات العشوائية الآتية : (A-01، A-02، A-03، A-04 ، A-05 ، A-06، A-07، A-08، A-09، A-10 و A-11، A-12).

أظهرت البادئات تعددية شكلية بين انواع أسماك الببغاء قيد الدراسة و سجلت كل البادئات حزم مؤشرة فريدة مختلفة تتراوح من ٢٠٠-٢٠٠٠ زوج قاعدة.أعطت البادئات المستخدمة مائة و احدى عشر (١١١) حزمة منها (١٣) حزمة أُحادِيُّ الشَّكْل (Monomorphic) و ثمان و تسعون (٩٨)) متعددة الشكل (polymorphic) مع معدل تباينى متعدد بنسبة ٨٨.٢٩٪ وكان أعلى معدل للتردد الحزمى لسمكة الببغاء من نوع *Scarus frenatus* مقارنة بالآسماك المدروسة.

وفقا لمصفوفة معامل البعد الوراثي (معامل Nei ٧٢) والتحليل العنقودي باستخدام طريقة المجموعة الزوجية غير الموزونة مع المتوسط الحسابي (UPGMA)، متوسط الفروق للصفات باستخدام انضمام الجار (NJ) و كذلك تحليل الإحداثيات الرئيسية (PCOA) وضح الآرتباط الوراثى بين الاسماك المدروسة والتى شكلت مجموعتين مع وجود ارتباط وراثي وثيق بين افراد كل مجموعة.

طبقاً للتحليل الكلاديستيكي (Cladistic) فان الحزم العشوائية التي تم الحصول عليها بواسطة RAPD تكون مفيدة لدراسة و فهم العلاقات التطورية. تلك الدراسة وضحت ارتباط أسماك الببغاء قيد الدراسة مع بعضها البعض، و وجود علاقة تطورية وثيقة بين انواع *Scarus frenatus*، *Scarus sordidus*، و *Scarus niger*.

انفصال سمكة *Scarus collana* كمجموعة قاعدية مستقلة ربما يدل غلى وجود سلف مشترك أمتلك صفات مميزة و موروثة في أسماك الببغاء التى درست.لقد وفرت تلك الدراسة معلومات ربما تكون مفيدة حول التباين الوراثي والعلاقات التطورية بين أسماك الببغاء.

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